1 DEHALOGENATION OF 2,4-DICHLOROPHENOXYACETIC ACID BY MEANS OF

2 BIOELECTROCHEMICAL SYSTEMS

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ABSTRACT

In this work, the dehalogenation of 2,4-D, has been studied by using two novel treatments for based on bioelectrochemical systems (BES): the Microbial Fuel Cell (MFC) and the Microbial Electrolysis cell (MEC). Both technologies demonstrated the feasibility of the dehalogenation of the 2,4-D molecule on an abiotic cathode. From the results, it was observed that, once achieved the steady state different performances were observed. The MFC spontaneously operated generating a current of around 500 mA m⁻² and a dechlorination of 10.14%. However, a better performance was obtained operating the MEC, in which 0.6 V were applied between anode and cathode, attaining a far higher dechlorination of 85.99% of 2,4-D in 4 days and a higher current of around 1700 mA m⁻².

Keywords

Bioelectrochemical system; herbicide; 2,4-Dichlorophenoxyacetic acid; dechlorination; reductive dehalogenation.

1. INTRODUCTION

Some pesticides and herbicides, chemically classified as chlorinated hydrocarbons, are characterized by their antimicrobial properties (Boye, Brillas, & Dieng, 2003). Their extended use and its toxicity generate an environmental problem of major concern (B. Carboneras, Villaseñor, & Fernandez-Morales, 2017; Chowdhury, Pradhan, Saha, & Sanyal, 2008). Amongst others, the 2,4-Dichlorophenoxyacetic acid (2,4-D) herbicide is a good model of these chemical compounds, widely used in the literature to evaluate the capabilities of different technologies to remediate soil and/or wastewater. 2,4-D is a polar molecule, slightly volatile and globally used in agricultural activities to avoid the growth of grassy weeds in crops, increasing therefore their productivity (B. Carboneras et al., 2017).

Many techniques have been investigated to treat these organochlorinated compounds. On the one hand, biodegradation is a relative low-cost process for the removal of organic compounds. Nevertheless, the number and position of chlorine groups on the aromatic ring increases significantly their bio-refractoriness and toxicity (Gallego et al., 2018; Sahinkaya & Dilek, 2006), making difficult the removal of these pollutants by means of bioprocesses. In the literature, the biodegradation by selected microbial species or microbial consortia has been studied. However, the low biodegradability and antimicrobial properties make the acclimatization of microorganisms an excessively long process, requiring also very long hydraulic retention times (B. Carboneras et al., 2017; M. B. Carboneras, Villaseñor, Fernández-Morales, Rodrigo, & Cañizares, 2018).

On the other hand, advanced electrochemical oxidation processes, such as electro-Fenton, combined with biological treatment (Dixit, Tirpude, Mungray, & Chakraborty,

2011; Huang, Liao, & Lai, 2013) and other physico-chemical methods (Alexander & Ramírez-Cortina, 2016; Van Aken, Van den Broeck, Degève, & Dewil, 2015) have also been reported in literature for the oxidation of chlorinated organics. Amongst the physico-chemical methods, it must be highlighted the use of Zero Valent Iron (ZVI). This technique is based on the capability of Fe^0 to dehalogenate chemicals (Correia de Velosa & Pupo Nogueira, 2013; Jia & Wang, 2012; Mukherjee, Sinha, Lama, & Kumar, 2015; Vidal et al., 2018).

Alternatively, some studies focus on the dehalogenation of the organochlorinated compounds through electrochemical technologies (Arellano-González, González, & Texier, 2016; He et al., 2016; Song, Lin, He, & Chen, 2009; Z. Sun, Wei, Hu, Wang, & Shen, 2012; Zhu, Baig, Xu, Sheng, & Xu, 2012). One of the advantages of dechlorinating chlorinated hydrocarbons is that the resultant by-products are less toxic and less bio-refractory, so a conventional biodegradation process may be set after this reductive process, in order to degrade entirely the resultant organics.

It has also been reported the coupled use of biological and electrochemical technologies for environmental remediation. These technologies are known as Bioelectrochemical Systems (BES) (Pant, Mohanakrishna, & Srikanth, 2015). BESs are systems that seize the catalytic capability of electrogenic microorganisms to convert the chemical energy of an organic fuel into electric energy in microbial fuel cells (MFCs), or on the contrary, harvest the electric energy to produce hydrogen/chemical products in microbial electrolysis cells (MECs), or other products formed at the cathode by an electrochemical reduction process. As compared to conventional electrochemical systems, BESs operate under relatively mild conditions, deal with a

87 wide variety of organic substrates and mostly do not use expensive precious metals as
88 catalysts (Pant et al., 2015).

89 In this context, one of the newest applications of BES technology is the treatment of
90 organochlorinated pollutants by both: the anodic oxidation (Pham, Boon, Marzorati, &
91 Verstraete, 2009; Zhang et al., 2011) and the cathodic reduction/dechlorination.

92 Several chlorophenol dehalogenation studies have been described in the literature
93 using BESs (Aulenta et al., 2007; Aulenta et al., 2010; Gu, Zhang, Li, & Lei, 2007;
94 Strycharz et al., 2010; Wen et al., 2013), showing their feasibility for the cathodic
95 reduction of organochlorinated pollutants, consuming less energy and using cheaper
96 electrode materials compared to conventional electrochemical systems (Wang, Luo,
97 Fallgren, Jin, & Ren, 2015).

98 Based on the aforementioned advantages and recent studies of BESs, the present
99 study aims to investigate the feasibility of dehalogenating the aromatic
100 organochlorinated herbicide 2,4-D at the abiotic cathode of BESs (working both under
101 MFC or MEC system), and to study its spontaneity, energy requirements and
102 efficiencies. This study also compares the results obtained by other conventional
103 electrochemical reduction techniques for eliminating 2,4-D. To the knowledge of the
104 authors, there are not previous studies using BESs to dechlorinate 2,4-D so far.

105

2. MATERIALS AND METHODS

2.1. Experimental set-up

The set-up used in this work consisted in two bench-scale BESs: a MFC with an external resistance of 50.8 ohm and a MEC, with an energy supply of 0.6 V between anode and cathode. Each cell consisted of two chambers (4 cm³ volume each) separated by a proton exchange membrane, PEM (Nafion-117, DuPont). The cells are formed by two phenolic resin plates, due to its chemical resistance, and two silicon plates to improve the mechanical properties and avoid liquid leakages, similar to the ones previously described by (Asensio et al., 2017). Carbon felt (KFA10, SGL Carbon Group®) was used as electrode material. Electrodes were cylindrical with a diameter of 2.5 cm and width of 1.5 cm. Catholyte and anolyte were stored in separate reservoirs of 120 mL and recycled into the cell by means of a Heidolph 5201 multichannel peristaltic pump. Electrochemical data were monitored by a Keithley 2000 multimeter connected to the BES. Figure 1 shows a scheme of the experimental set-up and a scheme of the dechlorination BES reactor.

Figure 1. (a) Scheme of the experimental set-up. **(b)** Conceptual description of the dechlorination BES reactor.

2.2. Anolyte and catholyte composition

A polluted wastewater with 300 ppm of 2,4-D was artificially prepared and used as catholyte. Additionally, a microbial growth medium was used as anolyte. The amounts of each chemical used in the anolyte medium formulation are presented in Table 1. Nutrients and trace elements, purchased from Sigma-Aldrich, were of analytical grade. The anodic fresh medium has a pH around 7.25 and conductivity of 4.5 mS cm^{-1} .

Table 1. Concentration of the organic source and nutrients of the anodic medium

Compound	Concentration (g L^{-1})
CH_3COONa	1
Na_2HPO_4	2
KH_2PO_4	0.5
$(\text{NH}_4)_2\text{SO}_4$	0.8
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2
CaCO_3	0.02
$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$	0.04

2.3. Start-up and stationary operation

The enrichment procedure and isolation of electrogenic consortium in the bio-anode was started with activated sludge inoculation from the Wastewater Treatment Plant of Ciudad Real, Spain. More information about this facility can be found elsewhere (Rodríguez Mayor, Villaseñor Camacho, & Fernández Morales, 2004). The anodic reservoir was filled with activated sludge and it was replaced the 50 % of the volume the next 4 days by new activated sludge with 1 g L⁻¹ of sodium acetate. Both anodic and cathodic reservoirs were connected to the respective anodic and cathodic chambers in the bioelectrochemical cell and maintained in continuous recirculation. Besides, both reservoirs were kept under anaerobic conditions, purging the anolyte and catholyte with nitrogen gas at the beginning of each batch cycle when changing the media. The anolyte and the catholyte stored in the reservoirs were homogenized by means of a magnetic stirrer. The homogenized liquid bulks were then pumped into the cell, at a flow rate of 4.7 mL min⁻¹, and returned to the reservoir tanks, operating therefore under sequential batch reactor operation mode. Catholyte was operated and pumped with the same conditions that for the anode. BESs operated under stationary semi-continuous mode at 24 ± 2 °C, performing batch cycles of 5 days. At the beginning of every stationary cycle, 80 % of the anolyte was replaced by fresh medium, and the catholyte was completely replaced by a new solution containing 300 ppm of 2,4-D.

2.4. Sampling and analytical and electrochemical measurements.

Regarding analytical tools, pH and conductivity were determined with GLP22 Crison pH meter and a Crison Cm 35 conductivity meter, respectively.

161 Samples from the catholyte for the measurement of 2,4-D concentration were taken
162 every day. The volume extracted was 1 mL per sample in order to avoid significant
163 changes in the total volume of the catholyte. 0.5 mL of the sample was used for the
164 high-performance liquid chromatography (HPLC) determinations, whereas the other
165 0.5 mL were diluted 10 times and then measured in the ionic chromatographic
166 determinations.

167 Regarding to the evolution of the 2,4-D concentration, it was determined by means of
168 HPLC with a UV detector (Jasco, Japan). The mobile phase consisted of 30:70 v/v
169 acetonitrile/0.1% phosphoric acid (flow rate of $1 \text{ cm}^3 \text{ min}^{-1}$). The injection volume was
170 $20 \mu\text{L}$ and the UV detection wavelength was 280 nm.

171 The rising of Cl^- was determined by ion chromatography using a Metrohm 930
172 Compact IC Flex coupled to a conductivity detector. A Metrosep A Supp 7 column was
173 used to determine the anions using a mobile phase consisting of 85:15 v/v 3.6 mM
174 Na_2CO_3 /acetone at a flow rate of $0.8 \text{ cm}^3 \text{ min}^{-1}$.

175 Regarding the electrochemical characterization, the electrical current produced by the
176 BESs was recorded every 5 minutes by means of a Keithley 2000 multimeter.

177 Polarization and power curves were preformed manually, changing the external
178 resistance. Before the measurements, the BESs were left in Open Circuit Voltage (OCV)
179 conditions for 2 h by removing the external resistor. Cyclic voltammetry (CV) tests for
180 the catholyte were performed on an Autolab potentiostat/galvanostat (PGSTAT-302N)
181 at scan rates of 100, 50 and 10 mV s^{-1} . The carbon cloth cathode electrode was used as
182 working electrode, pure Pt was used as counter electrode and Ag/AgCl electrode as
183 reference electrode in a half-cell reactor.

184 Once reached the steady state, the microorganisms supported at the anodic biofilm
185 were identified using a MALDI TOF mass spectrometer (AXIMA-Assurance supplied by
186 Shimadzu, Germany) according to the procedure described in the literature (S. Mateo
187 et al., 2016).

3. RESULTS AND DISCUSSION

3.1 Cathodic dehalogenation

To elucidate the possible reductive pathways of 2,4-D in the range of the voltage established, a cyclic voltammetry was performed (Fig. 2). Three voltage rates were set: 0.01, 0.05 and 0.10 V s⁻¹. From the results presented in Fig. 2, it can be highlighted one peak for the reduction reaction and another one for the oxidation in the different voltage rates and a very significant difference between the scans, which may be explained in terms of the occurrence of other less important oxidation and reduction processes (which may be explained because of the presence of a variety of organics and inorganic specie) or, most likely, by the significance of the capacitance of the double layer.. Thus, the increase of wideness of the cycles with the scan rate gives an idea of how capacitive the carbon felt electrode is. Regarding the peak, initially, in considering that the oxidation of sodium acetate to HCO₃⁻ at bioanodes with electroactive bacteria occurs at a potential -0.496 V (Ag/AgCl 3M KCl) and the reduction peak of the pesticide at the lowest scan rate is observed in the nearness of this potential, it can be hypothesized that this redox couple is thermodynamically feasible.

Comentado [MARR1]: No es cierto. Estas proponiendo un cambio que implica la transferencia de muchos electrones y con una voltametría solo ves una transferencia de uno o dos electrodos por pico!!!!!! Hay otros datos importantes: donde empieza, se hizo de derecha a izquierda o viceversa... Como mucho el pico podría indicar deshalogenación, pero eso debiese verse en posteriores barridos por que apareciese pico de oxidación de cloruros... Tenemos que ver despacio esta voltametría y si hay algun resultado voltamperométrico más

Comentado [FJFM2R1]: El lunes hablamos sobre esta gráfica

Comentado [MARR3R1]: Son muchos electrones para un solo pico....

Figure 2. Cyclic Voltammetries performed to 300 ppm of 2,4-D solution, varying the scan rate. Ag/AgCl reference electrode. Supporting electrolyte of Na₂SO₄, 70 mM.

Because of the thermodynamic feasibility of the redox couple, it was decided to start up simultaneously a MFC (without supply of external energy) and a MEC (which does have external energy supply). The MEC set-up was used with the aim of increasing the driving force and it was decided to apply 0.6 V in the MEC.

Both MFC (50.8 ohm) and MEC (0.6 V applied) were started up for long term operation. After three weeks, comparable cycles were attained in the systems. Current was obtained in the MFC as expected, confirming the spontaneity of the process.

Once reached similar performances in successive batch cycles (comparable current density and 2,4-D removal) a polarization curve was carried out on the MFC, in order to select the optimum operational conditions for the removal of the herbicide. The results obtained are presented in Figure 3.

Figure 3. Polarization curve and power curve of the MFC. Lines corresponds to the fitting of the model.

As can be seen in Figure 3, the OCV value reached was 640 mV, the maximum power density 53.57 mW m⁻², and the maximum current density of 328.4 mA m⁻². Since the objective is to favor the dechlorination rate of 2,4-D, it was decided to work in the region of the curve where a higher current is achieved, keeping the same small load with which this system was started up (50.8 ohm). The higher the current attained in the cell, the higher the speed of the overall process, and therefore, it may increase the consumption rate of 2,4-D. The high flow rate set by the peristaltic pumps attempts to minimize the losses of mass transfer.

Attempting to go deeper on the behaviour of the MFC, the polarization curve was fitted to an empirical model proposed by (Kim, Jung, Regan, & Logan, 2007).

$$E_{cell} = E_0 - b \cdot \log(i) - R \cdot i - m \cdot \exp(n \cdot i) \quad [1]$$

In the model, the term $-b \cdot \log(i)$ represents activation losses, $-R \cdot i$ the ohmic losses, and $-m \cdot \exp(n \cdot i)$ is an empirical term which represents mass transfer losses. Then, a polarization curve may present three stages related to these three losses. Depending on the system, one of the stages may have a greater importance over the others.

Precise initial approximations are required for accurate fitting. The initial approximation of the E_0 was based on the OCV value measured in the polarization curve. The parameter b was supposed to be -0.005 V/decades; this value is in agreement with the typical values presented in previous works (S. Mateo, Cañizares, Rodrigo, & Fernandez-Morales, 2018). Finally, initial values for the m and n parameters were taken from the literature (Hao, Shen, Hou, Zhou, & Wang, 2016). Once selected the initial approaches, all the parameters were simultaneously fitted to the experimental results presented in the polarization curve. The fitting values of all the parameters, as well as the obtained results, are presented in Table 2 and in Figure 3.

245

246 **Table 2.** Fitting values of the parameters of the empirical model corresponding to the
247 polarization curve.

Parameter	Value
E_0 (V)	0.563
b (V/decades)	0.008
R (Ω)	1.491
m (A)	7.31E-06
n (A^{-1})	27.705
r^2	0.99792

248

249 Figure 4 shows a chronoamperometry of both BES (a batch cycle, from day 60 to day 62
250 since start-up). It may be seen in Fig.5 a slight drop in the current achieved by the MFC
251 from the half to the end of the batch cycles, due to the depletion of reactants. In the
252 MEC, the current remained practically in the same value, probably due to the promotion
253 of non-desirables reactions, such as the production of H_2 at the cathode. The
254 chronoamperometry shows a better performance and higher current achieved by the
255 MEC, because the applied voltage act as a driving force.

256

257

258 **Figure 4.** Chronoamperometry of the BES. Batch cycle from day 60 to day 62 since
259 start-up.

260 Samples were taken every day during some of the batch cycles, in order to study the
261 removal of 2,4-D and the release of chloride anions. Average concentration values of 3
262 steady state cycles are presented in Figure 5.

263

264 **Figure 5.** Removal of 2,4-D (continuous line) and generation of chlorides (dashed line)
265 in the cathode; **a)** MFC; **b)** MEC

As can be seen in Figure 5, the dehalogenation of the organochlorinated compound implied an increase in the chlorides concentration in the liquid bulk of both systems, confirming the initial hypothesis. In the MEC, in which 0.6V were supplied between anode and cathode, a perfect correlation between the 2,4-D removal and the release of chlorides can be observed. At the end of every batch cycle, almost all the 2,4-D was dehalogenated.

Besides, it was calculated the theoretical amount of chlorides released considering the total dechlorination of the herbicide, from an initial concentration of 300 ppm of 2,4-D, resulting in 96.23 ppm of Cl⁻. The total dehalogenation of the molecule is being carried out effectively as it may be seen in Fig. 6.b, attaining an average dechlorination of 85.99 % at the end of the batch cycles in the MEC.

To understand better the behavior of the two systems, they were analyzed and kinetically modelled.

Regarding the MFC, the fitting to a first order kinetics was carried out. The controlling stage in this system was considered to be the electrochemical reaction taking place at the cathode [Eq.1].

$$-r_A = K \cdot C_A \therefore -\frac{dC_A}{dt} = K \cdot C_A \therefore C_{2,4D} = C_{2,4D_0} \cdot e^{-Kt} \quad [1]$$

Likewise, a correlation with the generation of chlorides was developed assuming a total dechlorination of the molecule, that is to say, that every molecule of 2,4-D dehalogenated gives two chlorides (this assumption is justified below). Note that total dechlorination of 300 ppm of 2,4-D would give 96.23 ppm of chlorides [Eq.2].

$$-\Delta C_{2,4-D} = \Delta C_{Cl^-} \cdot \frac{96.23}{300} \therefore -(C_{2,4-D}^t - C_{2,4-D}^{t_0}) = (C_{Cl^-}^t - C_{Cl^-}^{t_0}) \cdot \frac{96.23}{300}$$

$$C_{Cl^-} = 300 - C_{2,4-D} \cdot \frac{96.23}{300} \quad [2]$$

289 The fitting parameters and the results of the fitting are shown in Table 3 and Figure 6.

290

291 **Table 3.** Fitting values of the parameters of the first order model.

Parameter	Value
$K / \text{mg L}^{-1} \text{h}^{-1}$	$8.5 \cdot 10^{-4}$
r^2	0.7170

292

293

294 **Figure 6.** Modelling of the depletion of 2,4-D and generation of chlorides. Lines

295 indicates the model fitting.

296

297 In order to describe the MEC behavior, a mathematical model of the controlling stage,
 298 the biological one, was proposed. This mathematical model was based on the Monod
 299 equation and described the dehalogenation of the 2,4D [Eq 3].

$$r_s = \frac{dS}{dt} = -\frac{1}{Y} \cdot \mu_{max} \cdot \frac{S}{K_S + S} \cdot X = -K \cdot \frac{S}{K_S + S} \quad [3]$$

300

301 Where μ_{max} is the maximum growth rate (mg/d·gVSS), K_d is the decay coefficient (1/d),
 302 Y is the yield coefficient (g/g), X is the concentration of microorganisms (g/L), S is 2,4-D
 303 concentration (mg/L) and K_S is the half-saturation coefficient (mg/L).

304 For this model, it was transformed the differential term into finite increments, as
 305 follows.

$$306 \quad \frac{dS}{dt} \approx \frac{\Delta S}{\Delta t} = -K \cdot \frac{S}{K_S + S} \therefore S_t = S_{t-1} - K \cdot \frac{S}{K_S + S} \cdot (t_t - t_{t-1})$$

307 The growth of microorganisms at the biofilm from the beginning to the end of each
 308 electrolysis cycle was estimated as follows:

$$309 \quad Y = \frac{dX}{dS} \therefore Y = \frac{\Delta X}{\Delta S} \therefore X_t = \Delta S \cdot Y + X_{t-1}$$

310 By using the value of the steady state biomass concentration and assuming a
 311 biomass yield of 0.65 for the acetate degradation it was possible to estimate the
 312 concentration of biomass along the experiments.

313 In order to determine the biodegradation parameters, the mathematical model
 314 describing the pesticide removal and the biomass growth was fitted to the
 315 experimental data set obtained in the batch experiments. An accurate estimation of
 316 the model's parameter requires an initial set of values. These initial values were taken

from literature (Sara Mateo, Cañizares, Rodrigo Manuel, & Fernandez-Morales Francisco, 2018) and should guarantee the convergence of the mathematical functions to obtain a minimal squared error. The initial estimates $\mu_{\max}$ 0.06 1/d, K_s 1 mg/L and Y 0.65 g/g. Moreover, a correlation with the generation of chlorides was carried out as indicated previously. The obtained results are presented in Table 4 and in Figure 7.

Table 4. Fitting values of the parameters of the biological model.

Parameter	Value
$K / \text{mg h}^{-1} \text{mg}^{-1}$	9.298E-3
$K_s / \text{mg L}^{-1}$	80
$Y / \text{mg mg}^{-1}$	0.65
r^2	0.9917

Figure 7. Modelling for the MEC of the depletion of 2,4-D (a) and generation of chlorides, calculation obtained from the previous fitting.

3.2 Electrical performance

The electrical performance was studied in each device in order to evaluate their efficiency. Since the organic substrate in the anode was in excess in order not to be the limiting reagent, electrical efficiencies were expressed in terms electrons consumed to reduce 2,4-D, calculated as shown in the next expression for discontinuous operation mode:

$$\eta_c = \left(\frac{\int_t^{\Delta t} I dt}{n \cdot F \cdot \Delta C_{2,4D} \cdot V_{cat}} \right)^{-1}$$

where n is the number of electrons that are involved in the electrochemical reaction per mol of 2,4-D; F the Faraday's constant ($96485.3 \text{ C mol}^{-1}$ electrons); $\Delta C_{2,4D}$ is the molar consumption of 2,4-D during Δt , in mol L^{-1} ; V_{cat} is the volume of the catholyte (L) and I is the current intensity (A).

Thereby, an average electrical efficiency of every batch cycle was calculated, being 5.14 % for the MFC and 10.37 % for the MEC, these low values obtained indicates that this BES system was more efficient. The higher efficiency reached in the MEC means that electrons were more intended for the dehalogenation of 2,4-D and not for other parallel reactions. Other possible parallel reactions are other reduction pathways of the molecule which does not give chlorides (Boyle, Knight, Häggblom, & Young, 1999; C. Sun et al., 2014; Tsyganok, Yamanaka, & Otsuka, 1998), the promotion of hydrogen or the reduction of oxygen which may be dissolved in the catholyte (although O_2 concentration was minimized purging both catholyte and anolyte at the beginning of each batch cycle). Once finished the experiments and opened the devices, the biomass attached to the anodic electrodes was gravimetrically determined, presenting very similar values both, the MEC and the MFC, being their values $1,81 \text{ g g}^{-1}_{\text{electrode}}$ and $2,12 \text{ g g}^{-1}_{\text{electrode}}$

respectively. Additionally, the consortia in the biofilm was characterized through mass spectrometry, being the microbial population those presented in Table 5.

Table 5. Microbial characterization of the biofilm through mass spectrometry.

Family	Genus	Species	% match
<i>IV Comamonadaceae</i>	<i>Comamonas</i>	<i>testosteroni</i>	87.1
<i>Comamonadaceae</i>	<i>Aeromonas</i>	<i>s.p.</i>	80.7

With regard to the microbial population identified, both bacteria were previously reported as electrogenic according to literature (Chen, Liu, Hung, Shiau, & Wang, 2013; Lu, Huggins, Jin, Zuo, & Ren, 2014; Nguyen, Hong, Park, Jo, & Lee, 2015; Yu, Wu, Cao, Gao, & Yan, 2015). Results presented in Table 5 indicate the highest match percentages in both BES systems. The different operating conditions in each BES system led to a biofilm dominated by *Comamonas testosteroni* in the MEC while *Aeromonas s.p.* was the dominating one in the MFC. However, both consortia were the most relevant at both anodic biofilms. Additional strains were observed but their identification was not reliable.

The feasibility of such BES technologies compared to conventional electrochemical dechlorination still needs to be deeper studied. According to previously developed in the introduction, there are several investigations on the dechlorination of chlorophenols based on BES (Aulenta et al., 2007; Aulenta et al., 2010; Gu et al., 2007; Strycharz et al., 2010; Wen et al., 2013), which provide good results of feasibility of this technology. In addition, these devices require less input of electric energy compared to conventional electrochemical reduction processes.

373 Regarding to 2,4-D, only few works about conventional electrochemical dechlorination
374 have been reported while no previous studies about bioelectrochemical dechlorination
375 were found. Table 6 shows a comparison between these works and the present one.
376 Although it is difficult to establish a comparison between the results of the present work
377 (using BESs) and previous works (pure electrochemical systems), because of the
378 different operational conditions, Table 6 presents this comparison. In this Table, the
379 operational conditions and the results obtained when using pure electrochemical
380 dechlorination of 2,4-D as well as the main results obtained in the present work using
381 BESs are presented.

382 Even though BES may be thermodynamically more favorable as compared to pure
383 electrochemical systems and bare electrodes (without catalyst load), many parameters
384 still need to be optimized (reactor architecture, electrolyte, electrodes) and a lot of work
385 is yet to be done as lower current densities were achieved and high retention time is
386 needed. Besides, the response of microbial activity to higher current densities may be a
387 limiting factor. However, since milder operating conditions were set, better current
388 efficiencies were attained compared to pure electrochemical systems with catalyst load
389 at the working electrode (overpotentials for H₂ evolution at carbon-based electrodes are
390 very significant). This technology may spend more time for a complete dechlorination
391 but is far less expensive seizing the catalytic activity of electrogenic consortia at the
392 anode biofilm instead of precious metals.

Table 6. Comparison of operating conditions between pure electrochemical dechlorination and this work.

Authors	Working electrode	Reactor	Initial concentration	Energy input	Time	Removal of 2,4-D (%)	Current efficiency (%)
(Tsyganok et al., 1998)	Bare carbon felt (3x3x0.3 cm)	Teflon-made two-compartment reactor	50 mM <> 11.052 g L ⁻¹	111 mA cm ⁻² (5.7 – 7.8 V)	4 h	0.7	0.05
	Palladium-loaded carbon felt (1.0 wt. %)	Teflon-made two-compartment reactor	(Catholyte: TAA 0.5M, TFAA 0.5M, MeOH 40% v/v)	111 mA cm ⁻² (1.8 – 2.1 V)	4 h	25	2.5
	Palladium-loaded carbon felt (12.1 wt. %)			111 mA cm ⁻² (1.2 - 1.5 V)	4 h 7 h	71 100	8.5
(Z. Sun et al., 2012)	Nickel foam, Pd loading (1.78 mg cm ⁻²)	two-compartment electrolytic cell	50 mg L ⁻¹ <> 0.226 mmol L ⁻¹	1.667 mA cm ⁻²	4 h	71	~6.4
(C. Sun et al., 2014)	nTiN doping content (2mg), Pd loading (0.44mg cm ⁻²)	two-compartment electrolytic cell inside	50 mg L ⁻¹ <> 0.226 mmol L ⁻¹	1.667 mA cm ⁻²	1 h	72.01	6.7 (at 2h)
					2 h	~100	

	Pd loading (0.44mg cm ⁻²)	a T-controlled bath (25±0.1°C)		1.667 mA cm ⁻²	2 h	57.13	3.7
(He et al., 2016)	Foam-nickel electrode; Pd loading (0.67 mg)	H-type cell	50 mg L ⁻¹ <> 0.226 mmol L ⁻¹	1.5 mA cm ⁻²	3 h	98	1.5
Present work	Carbon felt	Two-compartment reactor. Batch mode operation	300 mg L ⁻¹ <> 1.357 mmol L ⁻¹	0.6 V applied (~0.17 mA cm ⁻²)	100 h	85.99	10.67

394

395

4. CONCLUSIONS

Bioelectrochemical systems are feasible technologies for the cathodic dechlorination of 2,4-D. Even though the process was proved to be spontaneous with energy production in the MFC, the MEC showed a better performance dehalogenating 2,4-D. Almost all the herbicide in the catholyte was dehalogenated after batch cycles of 4 days, being the molecule completely dechlorinated. Such bioelectrochemical systems for 2,4-D dechlorination may be thermodynamically more favorable compared to pure electrochemical systems and high current efficiencies could be attained but additional effort is yet to be done as low current densities were achieved and high retention time were needed.

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Comentado [MARR4]: Muy importante!!!!!!

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